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# GABA and Glutamate: Their Transmitter Role in the CNS and Pancreatic Islets

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Additional information is available at the end of the chapter

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## Abstract

Glutamate and gamma-aminobutyric acid (GABA) are the major neurotransmitters in the mammalian brain. Inhibitory GABA and excitatory glutamate work together to control many processes, including the brain's overall level of excitation. The contributions of GABA and glutamate in extra-neuronal signaling are by far less widely recognized. In this chapter, we first discuss the role of both neurotransmitters during development, emphasizing the importance of the shift from excitatory to inhibitory GABAergic neurotransmission. The second part summarizes the biosynthesis and role of GABA and glutamate in neurotransmission in the mature brain, and major neurological disorders associated with glutamate and GABA receptors and GABA release mechanisms. The final part focuses on extra-neuronal glutamatergic and GABAergic signaling in pancreatic islets of Langerhans, and possible associations with type 1 diabetes mellitus.

**Keywords:** glutamate, GABA, CNS, pancreatic islets, neurological disorders autoimmune diabetes

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## 1. Introduction

Glutamate and gamma-aminobutyric acid (GABA) are the major neurotransmitters in the brain. Inhibitory GABA and excitatory glutamate work together to control many processes, including the brain's overall level of excitation. A balanced interaction is required to maintain the physiological homeostasis, while prolonged imbalance can lead to disease. Glutamatergic/GABAergic imbalance can be found in autism spectrum disorders and anxiety disorders with elevated glutamatergic neurotransmission, while high levels of GABA produce more relaxation and even sedation. Neurotransmitter levels can be affected by external factors, for example, alcohol. Alcohol potentiates the sedentary effects of GABA, while inhibiting the excitatory aspects of glutamate, resulting in an overall increase in GABA/glutamate ratio. This leads to

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sensations of relaxation and in later stages to loss of control with slurred speech, unsteady gait and loss of social anxiety. The GABA/glutamate balance can also be affected by autoimmunity and genetic disorders. The contributions of GABA and glutamate in extra-neuronal signaling are by far less recognized. We will discuss extra-neuronal GABAergic and glutamatergic signaling and its relevance in insulin secretion from the pancreatic islets of Langerhans.

## 2. Glutamate and GABA during development

Both glutamatergic and GABAergic neurons are highly diversified in the central nervous system (CNS). More than half a century after the discovery of the effects of GABA, it is now established that in mature neurons, neuronal excitability is characterized by a balance between glutamatergic excitatory input and GABAergic inhibitory transmission. This balance is reached during development. However, the functions of GABAergic signaling are not restricted to a pure inhibitory mechanism at the synaptic level. This is a too simplistic view. For instance, GABA influences patterns and oscillations that are very relevant from the behavioral point of view [1]. GABA and glutamate expression are already widespread in the embryonic stage, and glutamate receptors are expressed in neurons even before glutamatergic synaptogenesis [1]. While glutamate receptor activities tune the developing GABAergic synapse [2], GABA is now considered the main excitatory transmitter during early development, acting not only at a synaptic and network level, but also on cell cycle and migration [1]. This excitatory function of GABA is caused by elevated neuronal intracellular chloride concentration at the early stages of development. The efflux of chloride mediated by GABA in immature neurons is excitatory, triggering sodium spikes and activating voltage-gated  $\text{Ca}^{2+}$  channels [1]. With time, a progressive reduction of chloride efflux occurs. This explains the shift from a depolarizing to a hyperpolarizing effect. Obata and colleagues were the first to suggest this developmentally regulated shift at the level of the spinal cord [3]. Neuronal chloride homeostasis is regulated by channels, exchangers and co-transporters. The developmental changes of sodium-potassium-chloride cotransporter 1 (NKCC1) (ensuring chloride uptake; higher expression in immature neurons) and potassium-chloride transporter 2 (KCC2) (principal chloride extruder; higher expression in mature neurons) are the masterpieces for the changes in chloride efflux associated with maturation. The developmental shift from local to large-scale network activity occurs in parallel with a gradual shift from electrical to chemical synaptic transmission [4].

It is noteworthy that in immature neurons activation of  $\text{GABA}_A$  receptors leads to an increase in the intra-cellular concentration of  $\text{Ca}^{2+}$ , as a consequence of the stimulation of voltage-gated  $\text{Ca}^{2+}$  channels, which exerts trophic effects on neuritic growth, migration and synaptogenesis. Blocking the  $\text{GABA}_A$  receptor reduces the cytoplasmic concentrations of  $\text{Ca}^{2+}$  [5]. In addition, the activation of  $\text{GABA}_B$  receptors depresses the  $\text{GABA}_A$  receptor-mediated  $\text{Ca}^{2+}$  increase and therefore the  $\text{GABA}_B$  pathway is likely supervising the entry of  $\text{Ca}^{2+}$  [6]. In granule cells of the cerebellum, the changes in the concentrations of  $\text{Ca}^{2+}$  outlast the exposure to GABA by several minutes [7]. The  $\text{GABA}_A$ -activated  $\text{Ca}^{2+}$  influx regulates the expression of the chloride extruder KCC2 [8]. One example of the relevance of this physiological shift in the chloride gradient occurs during delivery when the maternal hormone oxytocin triggers labor [9].

The electrical activity of neurons is a guide for the genesis of neuronal connections. Indeed, neuronal activity exerts a key role in the development of inhibitory GABAergic synapses, interacting closely with genetic programs. Blocking neuronal activity in developing neurons decreases the density of inhibitory synapses, confirming an activity-dependent development [10, 11]. The expression of GABAergic plasticity is related to modifications in the quantity of neurotransmitter in individual vesicles. Migrating neurons express, already at an early stage, both GABA and glutamate receptors [1], but GABA receptors are likely to be established first [12]. Interestingly, tangentially migrating interneurons express AMPA but not GABA or NMDA receptors. Therefore, a modulation/targeting of neurons via selective activation of receptors can be achieved [13]. This has implications for understanding and treatment of migrating disorders affecting the nervous system.

The building of brain networks is a highly complex task which requires organized sequential events, both spatially and from a timing standpoint. While an overdrive of GABAergic signaling slows the development, the overactivity of glutamatergic signaling causes excitotoxicity [1]. GABA receptors are the first to be active, even when synapses are still non-operant. This creates a shunting effect preventing excitotoxicity, since the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  spikes triggered by GABA require only a 30–40 mV driving force. Such a shunt is part of the synergistic interactions between GABA and glutamate. Thanks to these interactions, the neuronal networks in development generate primitive patterns of discharges, observed *in vivo* and in cultured networks, such as the giant depolarizing potentials (GDPs) which allow the building of functional units [1]. GDPs resemble interictal-like epileptiform discharges and provide synchronous  $\text{Ca}^{2+}$  oscillations also contributing to the development of networks. GDPs rely on the release of GABA, glutamate, and glycine at the onset of synaptogenesis [14]. The synchronized activity is one of the factors controlling the phenomenon of maturation [11]. Synchronization is also achieved, thanks to gap junctions, intrinsic voltage-dependent conductance [15], and non-vesicular paracrine release of neurotransmitters [16].

The capacity of developing nervous system to generate spontaneous activity in absence of external stimulation is a remarkable feature that has been observed in particular in the retina, the cerebral cortex, the hippocampus, the cerebellum, the hindbrain, and the spinal cord [14]. Recent works highlight that network bursts are driven by AMPA pathways in terms of coordination, whereas the shaping of the dynamics of spiking activities is regulated by NMDA- and GABA-associated currents [17].

### 3. Glutamate and GABA in the mature mammalian brain

#### 3.1. Biosynthesis of glutamate and GABA: the glutamate/GABA-glutamine cycle

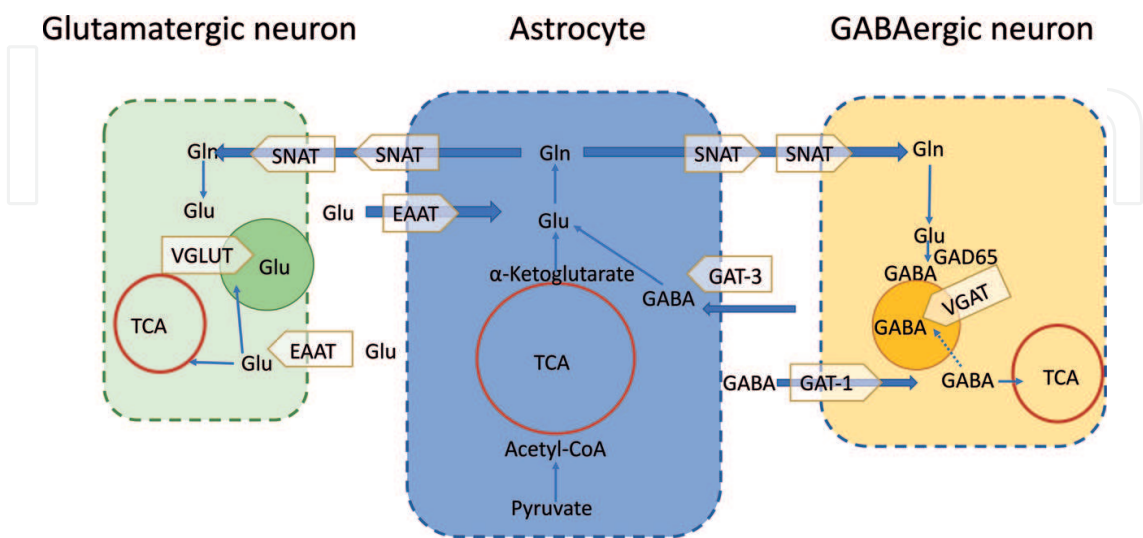
Glutamate and GABA do not cross the blood-brain barrier and must therefore be synthesized within the CNS. As neurons lack the enzyme pyruvate carboxylase and therefore cannot synthesize glutamate through the TCA cycle [18], they rely on astrocytes for the generation of glutamate. Astrocytes generate glutamate via *de novo* synthesis or by “recycling” glutamine from GABA and glutamate after reuptake. However, *de novo* synthesis makes up only ~15% of

astrocytic glutamate [19]. In this reaction, pyruvate is generated from glucose during glycolysis and enters the TCA after conversion to Acetyl CoA. The TCA product  $\alpha$ -ketoglutarate can be converted to glutamate, which is converted to glutamine by glutamine synthetase, an enzyme that is predominately, if not exclusively, located in astrocytes [20]. Glutamine exits astrocytes via the bidirectional N system transporters, SNAT3 and SNAT5 [21], and enter neurons via the unidirectional system A transporters, SNAT1, SNAT2 [21], and SNAT7 [22]. There glutamine is converted back to glutamate by phosphate-activated glutaminase, an enzyme which is expressed preferentially in neurons [23]. GABAergic neurons require an additional step to convert glutamate to GABA through decarboxylation. After release from the neurons, GABA and glutamate reenter the astrocytes to be “recycled” to glutamine. A small portion of glutamate is oxidatively metabolized, thus making *de novo* synthesis of glutamate necessary to maintain adequate glutamate levels [24]. The continuous recycling of glutamate, GABA and glutamine between neurons and astrocytes is known as the glutamate/GABA-glutamine cycle [25] (**Figure 1**).

3.2. Glutamatergic neurotransmission

In glutamatergic neurons, glutamate is packaged into synaptic vesicles (SVs) by vesicular glutamate transporters (VGLUT1–3) [26]. The loaded SVs then dock near the release site, where they are primed into a state of competence for  $Ca^{2+}$ -triggered fusion-pore opening. Once glutamate has been released, SVs can either fully collapse into the synaptic membrane, or close rapidly and undock (“kiss-and-run”) [27].

Released glutamate is recognized by glutamate receptors (GluRs). Binding of glutamate changes the receptor’s conformation and allows influx of extracellular  $Na^+$  and other cations, and an efflux of intracellular  $K^+$  ions. GluRs fall into two major categories: ionotropic and metabotropic [28]. Ionotropic GluRs are tetrameric ligand-gated cation channels that induce depolarization of the postsynaptic membrane. The three types of ionotropic GluRs are named



**Figure 1.** Overview of the glutamate/GABA-glutamine cycle. For details see text.



after ligands that selectively bind to one receptor only: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and Kainate, however all three are bound by glutamate [28]. NMDA receptors (NMDA-Rs) activate slower than AMPA and Kainate receptors. This delayed reaction is caused by the blockage of the cation pore of NMDA-Rs by external  $Mg^{2+}$  at resting membrane potential, which is removed upon depolarization of the neurons [29, 30]. Thus, non-NMDA-Rs activation is necessary to depolarize the neurons allowing NMDA-Rs activation. NMDA-Rs also close slower than non-NMDA-Rs, and therefore determine the duration of the synaptic current.

NMDA-Rs also allow influx of  $Ca^{2+}$  and have thereby a regulatory role in synaptic plasticity by connecting synaptic activity with  $Ca^{2+}$ -mediated biochemical signaling [31]. Depending on the nature of the neuron's depolarization, NMDA-Rs can both strengthen synapses, through long-term potentiation (LTP) [32, 33], and weaken synapses, through long-term depression (LTD) [34]. For LTP, repetitive and strong depolarization of the neurons allows significant influx of  $Ca^{2+}$  into the cytoplasm and activation of protein kinases, including the calcium/calmodulin-dependent protein kinase II (CaMKII), which (a) phosphorylate and activate AMPA-Rs and (b) trigger the insertion of additional AMPA-Rs into the postsynaptic membrane [35]. This increases the postsynaptic neuron's responsiveness to glutamate leading to LTP. By strengthening the neighboring connections of similar activity patterns, the NMDA-R enforces the Hebbian postulate that 'cells that fire together, wire together' [36]. For LTD, weak depolarization by low frequency stimulation still activates NMDA-Rs, but promotes only a modest prolonged increase in  $Ca^{2+}$  levels. Protein phosphatases (protein phosphatase 1 and protein phosphatase calcineurin) have a much higher affinity for calcium/calmodulin compared to CaMKII and are activated at lower  $Ca^{2+}$  levels. Thus, under the above conditions protein phosphatases are activated [37], dephosphorylate AMPA-Rs, and induce the removal of AMPA-Rs from the postsynaptic membrane [38], thereby reducing the postsynaptic responsiveness to glutamate and leading to LTD.

Metabotropic glutamate receptors (mGluRs) [39] are overall slower acting than iGluRs. In difference to iGluRs, mGluRs are not ion channels, but belong to a group of G-protein-coupled receptors. The associated G-protein consists of three subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ), of which the  $\alpha$ -subunit is associated with GDP. Glutamate binding to the receptor induces a conformational change that allows the replacement of GDP with GTP and consequent dissociation of the three G-protein subunits. While the  $G_{\beta/\gamma}$ -complex activates  $K^+$  and  $Na^+$  channels, the  $\alpha$ -subunit interacts with different enzymes [40]. Excitatory  $G_{\alpha}$ -subunits ( $G_{\alpha q}$  or  $G_{\alpha 11}$ ) bind and activate phospholipase C- $\beta$  (PLC- $\beta$ ), initiating a signaling cascade leading to the activation of protein kinase C (PKC) and the release of  $Ca^{2+}$  from the ER. Another excitatory  $G_{\alpha}$ -subunit ( $G_{\alpha s}$ ) activates the membrane-associated adenylyl cyclase, which catalyzes the conversion of ATP to cAMP, leading to activation of protein kinase A (PKA). Inhibiting  $G_{\alpha}$ -subunits ( $G_{\alpha i}$  or  $G_{\alpha o}$ ) prevent activation of adenylyl cyclase and the activation of PKA [41]. The three mGluRs groups (I–III) differ in their alpha subunits. Generally, Group I mGluRs carry activating  $G_{\alpha s}$  or  $G_{\alpha 11}$  subunits, are localized postsynaptically, and lead to cell depolarization and increased neuronal excitability. Groups II and III mGluRs carry inhibitory  $G_{\alpha i}$  or  $G_{\alpha o}$  subunits and are often localized on presynaptic terminals or preterminal axons, where they inhibit neurotransmitter release [42].

Released glutamate must be rapidly removed to avoid continuous stimulation and excitotoxicity [43, 44] (see also glutamate-induced excitotoxicity). Glutamate uptake is mediated via brain excitatory amino acid transporters (EAATs) on both pre- and postsynaptic neurons and on surrounding astrocytes [45]. Five subtypes of EAATs (EAAT1–5) have been cloned so far. A smaller portion of extracellular glutamate is reabsorbed by presynaptic or postsynaptic neurons.

Another mechanism involved in the extracellular glutamate regulation is the cystine/glutamate exchange transporter xc(–). In contrast to the EAATs, xc(–) is involved in elevating extracellular glutamate concentrations. Here intracellular glutamate is exchanged for extracellular cystine. The stimulation of xc(–) modulates glutamate release from the presynaptic neurons [46]. xc(–) regulates glutamate homeostasis through the involvement of the presynaptic mGluR2/3. Moreover, a decrease of xc(–) expression can lead to a reduction in extrasynaptic glutamate level. This effect may cause a loss of glutamatergic tone on presynaptic mGluR2/3, which can lead to a marked increase in glutamate release from presynaptic glutamatergic neurons [47].

### 3.3. GABAergic neurotransmission

For the synthesis of GABA, glutamate is decarboxylated to GABA by glutamate decarboxylase (GAD). GAD is expressed as two isoforms (GAD67 and GAD65) and can be found only in GABAergic neurons and in certain peripheral tissues, most prominently in the pancreatic islets [48]. GABA can also be taken up by presynaptic neurons after its release into the synapse. However, this recycled GABA is mainly metabolized to generate ATP through the GABA shunt pathway [49], while newly synthesized GABA is preferentially taken up into SVs [49]. Transport of newly synthesized GABA into SVs is tightly linked to its synthesis, as GAD65 and the GABA transporter VGAT form a protein complex with chaperone protein HSC70, the vesicular cysteine string protein (CSP), and CaMKII [49]. In the absence of GAD65 from this complex, the active site of VGAT may be available to cytosolic GABA, and vesicular transport of GABA can be restored to a certain extent [49]. GAD65 has also a crucial role in the trafficking of GABAergic vesicles to presynaptic clusters [50]. Palmitoylation of cysteine residues located at the N-terminus of GAD65 is required for the transport of GAD65 to synaptic terminals [51], and decreased palmitoylation impairs GABAergic neurotransmission, as observed in Huntington's disease [52]. Similar to glutamate, GABA is released in a  $\text{Ca}^{2+}$ -dependent manner upon depolarization of the presynaptic membrane.

GABA receptors present as ionotropic  $\text{GABA}_A$  receptors that cause rapid inhibitory postsynaptic potentials, and metabotropic  $\text{GABA}_B$  receptors that cause slow inhibitory postsynaptic potentials [53].  $\text{GABA}_A$  receptors are multi-subunit proteins [54] and consist of three major subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ). A total of five subunits are arranged around the central ion pore. The major receptor isoform consists of two  $\alpha_1$ , two  $\beta_2$ , and one  $\gamma_2$  subunits. GABA binding to  $\text{GABA}_A$  receptors on the postsynaptic neuron induces rapid and transient opening of  $\text{GABA}_A$  receptor  $\text{Cl}^-$  channels [55]. The subsequent influx of anions hyperpolarizes the membrane (phasic inhibition). GABA overspill can activate extrasynaptic  $\text{GABA}_A$  receptors, inducing a more prolonged opening of the ion channel (tonic inhibition) [56].

Metabotropic G-protein coupled GABA<sub>B</sub> receptors are mostly located extrasynaptically and can be found both pre- and the postsynaptically. These heterodimers consist of GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits. The GABA<sub>B1</sub> subunit can be bound by GABA, while GABA<sub>B2</sub> is coupled to the G-protein. Activation of GABA<sub>B</sub> receptors induces the dissociation of the subunits of the coupled G-protein. The G<sub>β/γ</sub>-subunit complex activates inwardly rectifying K<sup>+</sup> channels [57] and inhibits voltage-activated Ca<sup>2+</sup> channels [58], resulting in hyperpolarization of the neuron and inhibition of neurotransmission [59]. The G<sub>αi</sub>-subunit inhibits activation of adenylyl cyclase as described above for mGluR. Activation of presynaptic GABA<sub>B</sub> receptors impedes opening of voltage-activated Ca<sup>2+</sup> channels and thereby reduces neurotransmitter release. Thus GABA can inhibit its own release through a negative feedback loop via GABA<sub>B</sub> receptors present on GABAergic axons [60]. Activation of postsynaptic GABA<sub>B</sub> receptors reduces depolarization of the plasma membrane and thereby modulates excitatory signals.

The reuptake of GABA is mediated by GABA transporter protein present in presynaptic nerve terminals (GAT-1) and surrounding glial cells (GAT-3) [61].

### 3.4. Neurological diseases associated with glutamate and GABA receptors and GABA release mechanisms

#### 3.4.1. Limbic encephalitis

The relation between anti-GluR antibodies (Abs) with limbic encephalitis has been investigated during the last two decades [62, 63]. Several autoantibodies against extracellular epitopes of GluR involved in synaptic transmission and plasticity, such as AMPA-Rs [64] and NMDA-Rs [65] are described. The affected patients develop complex neuropsychiatric symptoms, such as memory deficits, cognition impairment, psychosis, seizures, abnormal movements, or coma. These disorders affect mainly young women, though cases of men and children have been reported [62]. Some of these patients present with malignant tumors and the syndrome can be qualified as paraneoplastic [62], characterized by association of anti-NMDA-RAb in ovarian teratoma and anti-AMPA-RAb in lung small cell carcinoma. Paraneoplastic limbic encephalitis can be fatal, but is curable if treated at an early stage by surgical removal of the tumor and a combination of immunotherapeutic agents [62]. Mechanistically, autoantibodies directed against AMPA-Rs and NMDA-Rs decrease the numbers of the cell-surface receptors [63] and anti-AMPA-RAb may act as agonists and increase cell excitability [66].

#### 3.4.2. Immune-mediated cerebellar ataxias

Compared with autoimmune encephalitis affecting the limbic system, autoantibodies to GluRs, such as anti-mGluR1 Ab and anti-glutamate receptor delta2 (GluRδ2) Ab, are less frequently associated with immune-mediated cerebellar ataxias (IMCAs). Anti-mGluR1Ab was reported in two patients with malignant lymphoma and one patient with prostate adenocarcinoma [67, 68]. Anti-mGluR1Ab impairs the induction of LTD, which causes ataxia in mice [67]. Interestingly, IMCAs associated with anti-GluRδ2Ab are always preceded or accompanied by either infection or vaccination [58]. Polyclonal Abs toward the putative ligand-binding



site of GluR $\delta$ 2 are known to cause AMPA-R endocytosis and attenuate their synaptic transmission, resulting in the development of ataxia in mice [69].

#### 3.4.3. Autosomal recessive cerebellar degeneration

Autosomal recessive CAs (ARCA) are affected by several gene mutations. One of these involves mutations in the GRM1 gene, which encodes mGluR1, known to play an important role in cerebellar differentiation [70]. Accordingly, the clinical features of this familial form of CAs appear already during childhood. The child shows global developmental delay, intellectual defects, severe CAs, and pyramidal signs. Brain imaging often shows progressive generalized cerebellar atrophy. Mutations affect a gene region critical for alternative splicing and the formation of the receptor structure.

#### 3.4.4. Glutamate-induced excitotoxicity

Deficits and mutations in GluR can also affect the level of extracellular glutamate with detrimental outcomes for neurotransmission and neuronal health. The level of extracellular glutamate is determined by three factors: (1) vesicular-released glutamate at the synapses, (2) non-vesicular-released glutamate from the system xc<sup>−</sup> (see above) [71], and (3) glutamate uptake by EAATs on astrocytes [72]. When glutamate release exceeds glutamate uptake, the excess glutamate activates a large number of postsynaptic NMDA-Rs, resulting in the induction of excitotoxic neuronal death by allowing excessive Ca<sup>2+</sup> influx through the receptor-operated cation channels [73]. Excessive activation of NMDA-Rs and the associated Ca<sup>2+</sup> influx result in stimulation of calpain I and nNOS [73]. This causes DNA damage and formation of ONOO<sup>−</sup>—due to excess NO (nitrosative stress) and other free radicals. The combination of these two changes ultimately leads to mitochondrial dysfunction and cell death [73]. Recent studies reported the presence of high glutamate levels in the extracellular space in chronic degenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease [74]. Taken together, glutamate-induced excitotoxicity may be a common process for neuronal death throughout the CNS and accelerates the original pathological changes.

#### 3.4.5. Neurological diseases associated with deficits in GABA receptors

Anti-GABA<sub>B</sub> receptor Ab is associated with limbic encephalitis [75], which manifests clinically similar to anti-GluRAB-mediated limbic encephalitis. Some patients also develop CAs. About 50% of the patients have neoplastic diseases, especially small cell lung carcinoma [75]. Clinical studies indicate that surgical removal of the lung tumor and subsequent immunotherapy can be effective in the relief of the above neurological disorders, especially in the early stages of disease.

#### 3.4.6. Association of GAD65 dysfunction with neurological disorders

Anti-GAD65Abs are associated with stiff-person syndrome (SPS) and CAs [76]. Titers of anti-GAD65Ab are high (>1000 U/ml) in both conditions. SPS is characterized clinically by progressive rigidity and painful-muscle spasms in the axial and limb muscles. Electromyography (EMG) shows simultaneous activities of the agonist and antagonistic muscles. Anti-GAD65Ab-associated

CAs affect mostly women in their 50–60s and exhibit subacute or chronic CAs, which are sometimes associated with SPS or epilepsy [76]. Furthermore, the majority of patients also suffer from type 1 diabetes mellitus (T1D) [76].

Based on the intracellular location of GAD65 (on the cytoplasmic side of SVs), the pathogenic role of anti-GAD65Ab in CA and SPS has been questioned. However, recent studies have shed new light on this issue. First, the pathogenic actions of anti-GAD65Ab have been clarified both in *in vitro* and *in vivo* preparations [77–82], for example, the Cerebrospinal fluid (CSF) of patients with SPS inhibited GABA synthesis [83]. Furthermore, SPS-like symptoms were reproduced in experimental rats and mice injected intrathecally or intraventricularly [84] or intracerebellarly [80] with IgGs obtained from the CSF of CA and SPS patients. Specifically, IgGs obtained from the CSF of patients with anti-GAD65Ab-associated CA depressed GABA release in cerebellar brain slices [77, 78, 82, 85], and their intracerebellar injection interfered with cerebellar control of the motor cortex and resulted in ataxic gait in rats [79]. These actions were reproduced by human anti-GAD65 monoclonal Ab b78, which binds to an epitope similar to that recognized in SPS patients positive for anti-GAD65Ab [80–82].

Second, studies in animals have shown internalization of antibodies by cerebellar neurons [81, 86], demonstrating that anti-GAD65Ab can access their intracellular target. Together, these results indicate the possibility that anti-GAD65Ab may damage sufficient numbers of GABAergic neurons to result in the appearance of frank neurological symptoms [87].

The pathological effects of anti-GAD65Ab depend on epitope specificity. Specifically, the pathologic effects of anti-GAD65Abs on GABA synthesis and release were specific to anti-GAD65Ab b78—representing SPS- and CA-associated anti-GAD65Ab—and could not be reproduced by monoclonal anti-GAD65Ab b96.11, representing an epitope specificity associated with type 1 diabetes mellitus [80–82]. This epitope dependence might explain the differences in neurological phenotypes. Administration of CSF obtained from patients with SPS or CAs reproduced the clinical features of the corresponding disease in mice [80]. Thus anti-GAD65Ab in SPS that inhibit GABA synthesis, would attenuate inhibitory Purkinje cell-mediated depression of the spino-cerebellar loop, resulting in increased muscle tone, whereas anti-GAD65Ab in CAs depress GABA release, resulting in disruption of Purkinje cell-mediated modulation of the cerebro-cerebellar loop to elicit disorganized movements [87]. An alternative explanation of the phenotypic differences is the involvement of other autoimmune-mediated mechanisms.

In anti-GAD65Ab-associated CAs, anti-GAD65Ab impairs the association of GAD65 with the cytosolic side of SVs resulting in a decrease in vesicular GABA contents with low release probability [82]. Under normal conditions, the released GABA spills over to the neighboring excitatory synaptic terminals and inhibits presynaptic glutamate release through GABA receptors. However, this GABA-induced inhibition of glutamate release is disturbed in patients with CAs [77]. Taken together, anti-GAD65Ab elicit marked imbalance in neurotransmitters; including a decrease in GABA and an increase in glutamate. The imbalance between GABA and glutamate is accelerated following the involvement of microglia and astrocytes [87]. Microglia activated by excessive glutamate levels can secrete various cytokines, which facilitate glutamate release presumably through xc(–) on microglia, and suppress the uptake of glutamate

through EAATs on astrocytes [71, 88, 89]. Thus, the neuroinflammation-induced chain reactions accelerate the imbalance, leading to profound excitotoxicity. In agreement with this notion, the cerebellar neurons are completely lost in patients with advanced stage CAs [90].

In conclusion, deficits in glutamate- and GABA-mediated synaptic mechanisms, upset the glutamate/GABA ratio. Notably, the level of glutamate is relatively high compared with that of GABA, caused by various etiologies, including glutamate release by damage-induced depolarization, exaggerated glutamate release through xc(-), and attenuated uptake of glutamate through EAATs, or a decrease in GABA release with subsequent increase in glutamate release. The imbalance between glutamate and GABA can trigger excitotoxicity, one of several neuron death mechanisms.

#### **4. GABA/glutamate signaling pathways in pancreatic islets and implications in type 1 diabetes mellitus**

While GABA and glutamate are best characterized for their role as neurotransmitter, they are also involved in extra-neuronal signaling. As a major building block in proteins synthesis, intracellular glutamate is abundantly present in the body. In contrast, GABA is present only in restricted non-neuronal tissues, including the pancreas [91]. Pancreatic islets are clusters of endocrine cells located in the exocrine pancreas and regulate blood glucose homeostasis. An islet typically contains insulin-releasing beta cells, glucagon-secreting alpha cells, somatostatin-containing delta cells, and pancreatic polypeptide-producing (PP) cells. The metabolic actions of insulin and glucagon are reviewed in great detail elsewhere [92]. Briefly, insulin is released at elevated blood glucose levels and acts as an anabolic hormone, causing cellular glucose uptake primarily in skeletal muscles, the liver, and fat tissue. Here glucose is converted to storable energy substrates including glycogen and triglycerides, respectively. At low blood glucose levels, glucagon is secreted from pancreatic alpha cells. Glucagon causes the liver to convert stored glycogen to glucose and induces lipolysis in fat tissue. Many regulatory mechanisms are in place to control the secretion of insulin and glucagon to maintain stable blood glucose levels. Within the islet, extracellular insulin inhibits glucagon secretion from alpha cells, while glucagon enhances both insulin and somatostatin secretion [93]. In recent years, GABA and glutamate have gained interest for their respective roles in the regulation of secretion of insulin and glucagon.

In the following sections, we will review GABAergic and glutamatergic signaling in the islets of Langerhans and possible implications for type 1 diabetes mellitus.

##### **4.1. Glutamate and GABA in pancreatic islets**

Both glutamate and GABA are synthesized from glucose taken up by beta cells. The majority of glucose is metabolized to produce energy, however a portion is converted to glutamate. The initial step is catalyzed by pyruvate carboxylase present in the beta cells [94]. Thus, in contrast to GABAergic neurons, beta cells can synthesize glutamate on their own. In the alpha cells, glutamate is loaded into glucagon-containing secretory granules via VGLUT1 and VGLUT2

[95]. Within the beta cells, glutamate is decarboxylated by GAD to yield GABA. GABA is packaged by the GABA transporter VGAT into small synaptic-like microvesicles (SLMVs) [96]. A smaller fraction of GABA is present in insulin-containing large dense core vesicles (LDCVs) [97, 98]. These vesicles also express VGAT, suggesting similar packaging mechanisms as for SLMVs [98]. GABA is present predominantly, if not exclusively, in the beta cells [96, 99, 100], and GABA and GAD expression levels in beta cells are similar to those in GABAergic neurons [97].

Both isoforms of GAD have been identified in pancreatic beta cells, GAD65 being the predominant isoform in rat and human beta cells, while mice beta cells only express GAD67 [48]. In human and rat beta cells GAD65 co-localizes with GABA at the SLMVs [101], while in murine pancreatic islets, GAD67 is firmly membrane anchored and efficiently transits to presynaptic clusters [102]. It remains unclear whether GAD is involved in SLMVs transport to the plasma membrane, in analogy to GABAergic neurons (see also Section 3.3).

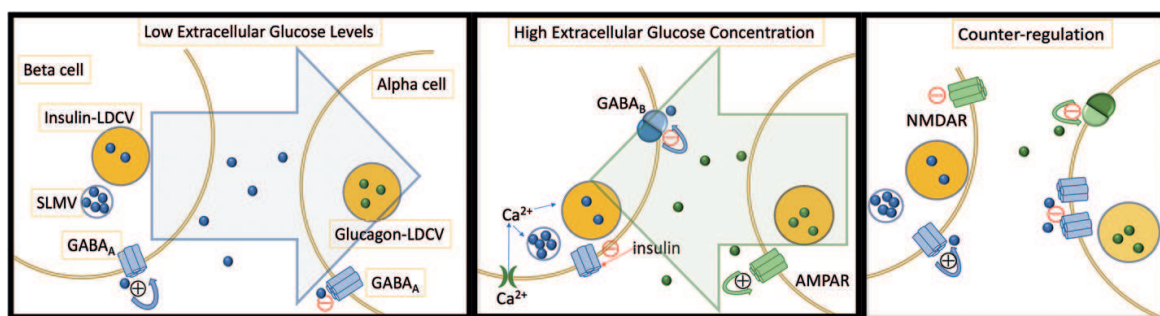
As outlined above, GABA is present in both SLMVs and insulin-containing LDCVs. The basal release of GABA from beta cells is relatively constant [101, 103], but can be modulated depending on the metabolic state of the cell [104]. The mechanisms involved in GABA release were first investigated in beta cell lines, where GABA secretion was shown to be dependent on the presence of extracellular  $\text{Ca}^{2+}$  [105], suggesting that GABA is released in response to an increase in cytosolic  $\text{Ca}^{2+}$  levels. A detection system for GABA release involving overexpressed  $\text{GABA}_A$  receptors in dispersed rat islets allowed the sensitive detection of GABA release as fluctuations in current in whole-cell patch-clamped beta cells [106]. These studies confirmed that GABA release is dependent on the entry of extracellular  $\text{Ca}^{2+}$  through voltage-gated channels and not by membrane depolarization itself. The study further indicated that the observed GABA release originated predominantly from SLMVs rather than LDCVs.

Both beta and alpha cells express  $\text{GABA}_A$  and  $\text{GABA}_B$  receptors [103]. Through the expression of GABA receptors on beta cells, GABA regulates its own secretion (autocrine regulation).  $\text{GABA}_A$  receptor activation induces further GABA release (autocrine positive feedback loop) [97, 107]. However, GABA-mediated regulation of GABA release depends on the extracellular glucose concentration. At high glucose concentrations, GABA hyperpolarizes the membrane of isolated beta cells and beta cell lines [108]. This inhibitory effects appears be mediated via  $\text{GABA}_B$  receptors [106, 109].

Presence of GABA receptors on adjacent alpha cells enables a paracrine regulation of these cells [110]. Activation of  $\text{GABA}_A$  receptors on alpha cells leads to hyperpolarization and suppression of glucagon and glutamate secretion [103, 111]. As glucagon and glutamate trigger insulin release from beta cells, GABAergic inhibition of glucagon and glutamate secretion indirectly downregulates insulin release from beta cells as a negative feedback regulation (**Figure 2**). Extracellular GABA is taken up by the plasma membrane GABA transporter GAT3 expressed on both alpha and beta cells [98].

At low concentrations of glucose, alpha cells show high action potentials [103, 112], mediated by voltage-gated  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels [113]. This triggers the release of glucagon and glutamate. At high glucose concentrations, release of glucagon and glutamate is inhibited,





**Figure 2.** Schematic outline of presumed GABAergic and glutamatergic signaling in pancreatic islets. At low glucose concentrations, beta cells release GABA in a glucose-independent manner [97, 108]. This tonic GABA release continuously activates GABA<sub>A</sub> receptors on beta cells and alpha cells. GABA<sub>A</sub> receptor activation leads to further GABA release from beta cells and suppresses release of glucagon and glutamate from alpha cells, thereby preventing insulin secretion. As extracellular glucose levels rise, the level of intracellular Ca<sup>2+</sup> increases in beta cells, allowing release of insulin. Secreted insulin inhibits GABA<sub>A</sub> receptors on the beta cells, thereby decreasing the autocrine positive feedback release of GABA from beta cells and reducing GABA-mediated inhibition of alpha cells [140]. Moreover, under high glucose concentrations GABA inhibits its own release from beta cells (via GABA<sub>B</sub> receptor activation). Reduced extracellular GABA concentrations allow glucagon and glutamate secretion from alpha cells. Glutamate triggers glutamate and glucagon release via activation of AMPA-Rs and glucagon stimulates further insulin release from beta cells. Counter-regulation: eventually, the depolarization of beta cells activates NMDA-Rs present on beta cells, initiating the repolarization of beta cells and limiting insulin release. Reduced insulin levels lift the inhibition of GABA<sub>A</sub> receptors on beta cells and allow positive feedback regulation of GABA release. Elevated extracellular GABA levels can now activate GABA<sub>A</sub> receptors on alpha cells and inhibit glutamate and glucagon release. Of note, insulin secretion induces GABA<sub>A</sub> receptors phosphorylation and translocation to alpha cell plasma membrane [111]. This renders alpha cells more susceptible to GABA-mediated suppression of glucagon secretion, and ultimately limits insulin secretion from beta cells [111, 141].

although the mechanistic details of this regulation remain unclear. Paracrine GABA-mediated regulation (as described above) is suggested by the finding that isolated rat alpha cells no longer show reduced glucagon/glutamate release at elevated glucose concentrations [112], and the observation that in rat islets and purified alpha cells, GABA antagonists suppress glucagon secretion [114]. Insulin and GABA are suggested to serve as paracrine inhibitors of glutamate and glucagon release [101, 103, 115].

Once released, glutamate activates GluRs expressed on both alpha and beta cells. The cell-specific distribution of AMPA-R/Kainate receptors and NMDA-Rs remains debated, and earlier reports suggested that AMPA-Rs are expressed exclusively on alpha cells, while NMDA-Rs were reported to be specifically expressed on beta cells [116]. However, later reports suggest that AMPA-Rs are also expressed on mouse beta cells, and other studies suggest iGluRs expression only on alpha and not on beta cells [117]. mGluRs mGluR8, mGluR5 and mGluR2/3 are expressed by beta cells [109, 118], while mGluR4 protein is expressed on alpha cells [119]. Extracellular glutamate activates AMPA/kainate Rs present on alpha cells and triggers the co-release of glutamate and glucagon (positive feedback regulation) [117, 120, 121]. On the other hand, activation of mGluR inhibits glucagon/glutamate secretion from alpha cells [119] (negative feedback regulation). Few studies reported that activation of AMPA/Kainate-R and/or mGluR on beta cells triggers insulin secretion [118, 120], however subsequent studies could not confirm these results [117]. Still, through glucagon-mediated insulin release from beta cells, co-secreted glutamate indirectly stimulates insulin secretion from beta cells [121]. In addition, glutamate stimulates GABA release from SLMVs in beta cells independently of insulin release,

thus serving as a regulatory factor to limit glucagon/glutamate release [106]. As in the GABAergic system, glutamate-mediated regulation of beta cells depends on the extracellular glucose concentration. The high-affinity NMDA-Rs on the beta cell are already saturated at physiological glutamate concentrations in the islet, and are mainly activated through depolarization of beta cells [121]. As islets are depolarized by external glucose, the NMDA-R-mediated repolarization of the beta cells is a negative feedback regulation of glucose-stimulated insulin secretion. Extracellular glutamate is taken up by EAAT1 and 2 expressed on alpha cells [122].

#### 4.2. Proposed GABAergic and glutamatergic signaling in type 1 diabetes mellitus

Type 1 diabetes mellitus (T1D) is an autoimmune disease, characterized by the specific destruction of pancreatic beta cells. Exogenous administration of insulin is necessary to avoid hyperglycemia. Additionally, within 5 years of disease diagnosis, almost all patients with T1D lose their ability to generate an adequate glucagon response to hypoglycemia [123]. This loss has been attributed to the lack of intracellular regulation of beta- to alpha-cell signaling during hypoglycemia [124] and may account for the elevated plasma glucagon levels in diabetes patients, indicating alpha-cell hypersecretion [125, 126]. As observed for neurons, beta cells are sensitive to elevated extracellular glutamate levels and show signs of secretory defects and apoptosis at high glutamate levels [122]. This effect was not prevented by AMPA-R and Kainate-R antagonists and therefore unlikely caused by excitotoxicity. Instead, oxidative stress appears to be the underlying mechanism of glutamate-induced beta-cell death. As outlined in detail in the CNS portion of this chapter, the glutamate/cystine antiporter system xc(−) exchanges intracellular glutamate for extracellular cystine. Excess extracellular glutamate inhibits and/or reverts the activity of the antiporter, thus depleting the cells of cysteine, a building block of the antioxidant glutathione, possibly increasing the cells' vulnerability to oxidative stress [122]. Upregulation of EAAT1 expression on beta cells protects beta cells from glutamate-induced toxicity [122], indicating glutamate signaling as a potential therapeutic target. Notably, many effective antidiabetic drugs such as GLP-1, exenatide, and glitazones also show significant neuroprotective activity against glutamate-induced cytotoxicity in the brain [127, 128]. Moreover, topiramate, an anti-epileptic drug that provides neuroprotection by preventing glutamate toxicity, has antidiabetic and beta-cell cytoprotective effects [129] and long-lasting remission was observed in a T1D patient after treatment with topiramate for generalized seizures [130].

GABA has an overall anti-inflammatory effect on the immune system [131]. GABA<sub>A</sub> receptors are expressed by T cells, B cells, and other mononuclear cells, and their activation suppresses lymphocyte proliferation [132, 133]. This GABA-mediated inhibition of T cell responses may provide the mechanism of GABA-associated protection of animal models for development of T1D [134]. GABA also promotes a shift from an inflammatory to an anti-inflammatory cytokine profile *in vivo* and *in vitro* [107]. Another aspect of GABA activity in regard to pancreatic beta cells has been only recently reported. Through activation of GABA<sub>B</sub> receptors, GABA significantly increases beta-cell viability [135] and replication [136]. In mouse models, GABA administration prevented and even reversed T1D [107]. One of the involved mechanisms may be GABA-mediated conversion of alpha cells to beta cells [137, 138]. While the details of the

mechanisms involved need to be further investigated, these studies open the intriguing potential to use GABA treatment to re-generate beta cells in T1D [139].

It remains unclear what may cause impaired GABAergic and glutamatergic signaling in the pancreatic islets. No mutations of receptors or other elements of the signaling mechanisms have been identified in T1D so far. GAD65Ab are present in the majority of patients with T1D and are regarded as a byproduct of the immune response without significant relevance for the disease progression. However, it is possible that in analogy to their role in neurological disorders, GAD65Ab are taken up by pancreatic beta cells and (a) uncouple the balanced regulation of insulin and glucagon secretion and (b) induce beta cell apoptosis through prolonged exposure to elevated extracellular glutamate levels. Further research is needed to determine whether GAD65Ab have a pathologic role in the development of T1D.

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